Dynamics of the Adsorption of Egg Albumin at the Silica–Solution Interface

A. K. BAJPAI* and R. DENGRE

Department of Chemistry, Government Autonomous Science College, Jabalpur 482 001 (M.P.), India

SYNOPSIS

The kinetic behavior of the adsorption of the egg albumin (EA) onto silica was studied from its alkaline aqueous solution at room temperature. Various adsorption and kinetic parameters such as the adsorption coefficient, surface coverage (θ), and rate constants for adsorption and desorption were evaluated using different rate expressions. The adsorption was found to increase on addition of H^+ ions to the protein solution. It was also observed that the presence of inorganic salts and cationic and anionic detergents influence both the adsorbed amount and the adsorption rate. The effect of temperature on the adsorption was also investigated. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Increasing use of biomedical materials for artificial blood vessels, hearts, kidneys, and other organs after they are implanted into a living body have stimulated great interst in studying the interactions between proteins and plastic, metal, and ceramic surfaces.[1-3] Considerable progress has been marked in the study of the kinetics of adsorption of certain proteins[4-6] onto various surfaces. However, much fewer investigations have been carried out for studying the dynamic aspects of the adsorption of food proteins.

From this laboratory, we have published results of several investigations on the adsorption of polymers,[7-10] proteins,[11,12] and sulfa drugs[13] onto various solid surfaces. In the same series, the present communication describes the results of the kinetics of the adsorption of egg albumin (EA) onto a silica surface from its aqueous solution.

EXPERIMENTAL

Materials

Egg albumin (MW 33,000 and isoelectric pH 4.6) in a flaky form was supplied by the Wilson Laboratories (Bombay, India) and used without further purification. The adsorbent silica was obtained from Loba Chemicals, India, and used without any pretreatment. The specific surface area of the adsorbent was reported as 22.0 $m^2\ g^{-1}$. Other reagents employed in experiments were of guaranteed reagent grade. All solutions were prepared in bidistilled water, and a fresh solution of EA was prepared for each run.

Method

The methods for carrying out adsorption and its kinetics have already been described in our previous communications.[7,8] In brief, a known volume of alkaline solution of EA (pH 11.6) along with a fixed amount of silica were shaken for 2 h to ensure the adsorption equilibrium to be attained and the amount of the adsorbed EA was estimated colorimetrically.[14]

RESULTS AND DISCUSSION

Concentration Effect and Adsorption Isotherms

The amount of the adsorbed EA is frequently found to approach a plateau value as the solution concentration is increased in the range from 1.53 to 9.18 $\times 10^{-5}$ mol dm^{-3}, which is consistent with the sat-

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 60, 2219–2225 (1996)
 CCC 0021-8995/96/122219-07

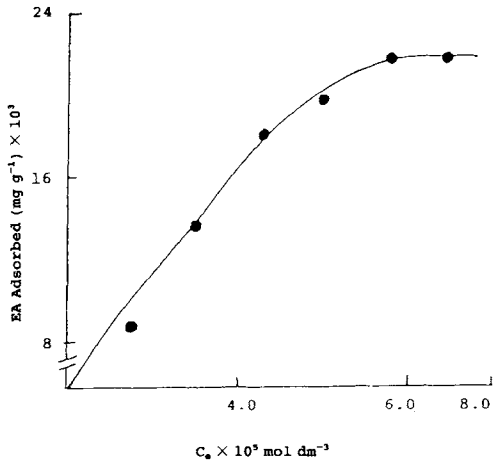

Figure 1 Amount of EA adsorbed (mg g^{-1}) vs. the equilibrium concentration of the protein solution (mol dm^{-3}).

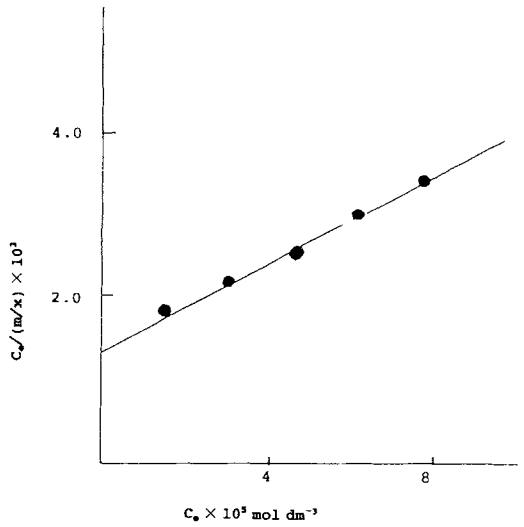

Figure 2 Plot between $C_e/m/x$ and equilibrium concentration C_e to evaluate K.

uration of available sites. Results are quite obvious, as with increasing concentration of the EA solution more and more EA molecules arrive at the silica solution interface and become adsorbed onto the surface. The adsorption isotherm so obtained is shown in Figure 1, where the amount of EA adsorbed (in mg g^{-1}) was plotted against the equilibrium concentration of the protein solution (in mol dm^{-3}). The shape of the isotherm belongs to the L2 type,[15] i.e., the Langmuir type, and has been widely found in protein adsorption experiments.[16]

To evaluate the adsorption coefficient K ($= k_1/k_2$), a plot is drawen between C_e/m and C_e according to the following Langmuir equation:

$$\frac{C_e}{m} = \frac{1}{K_1' K} + \frac{C_e}{K_1'} \qquad (1)$$

where $K = k_1/k_2$, k_1, and k_2 are the rate constants for the adsorption and desorption; K_1' = a constant; C_e = equilibrium concentration of the EA solution; and m = amount of EA adsorbed in mg g^{-1}. Such a plot in the present investigation is shown in Figure 2 and the numerical value of the adsorption coefficient was found to be 8.45×10^{-5} dm^3 mol^{-1}.

Surface Coverage (θ)

The surface coverage (θ) not only reflects the fraction of the available sites occupied by the adsorbate but also provides a parameter to show the progress of the adsorption process. To calculate θ values at different time intervals, eq. (4) of Ref. 8 was used, according to which

$$\theta = 1 - \frac{C + K'}{C_0 + K'} \qquad (2)$$

where $K' = 1/K$, C_0 = initial concentration of the EA solution, and C = concentration of the EA solution at time t.

The results obtained are shown in Figure 3, which clearly indicate that as the adsorption process proceeds the EA molecules continue occupying the active sites on the silica surface. In our previous studies also, we obtained a similar type of results.

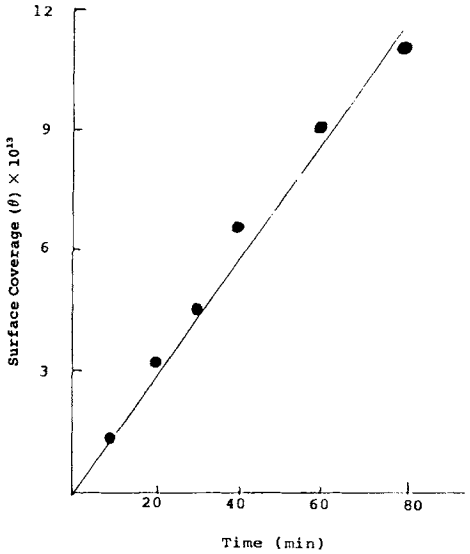

Figure 3 Variation of surface coverage (θ) with time at fixed [EA] = 6.15×10 mol dm^{-3}; silica = 0.1 g; pH 11.6; temperature = 25 ± 0.2°C.

Kinetics of Adsorption

Rate of Adsorption

It is well confirmed by experiments that the rate of adsorption of large molecular weight substances depends on (1) the transport or diffusion of mass toward the interface from the bulk, (2) the attachment of adsorbate molecules to the surface, and (3) reconformation of the adsorbing macromolecule. In the case of proteins, all the three steps are equally significant, as in a large number of protein adsorption experiments, the adsorption has been found to be diffusion-controlled and reconformation of the adsorbed protein has also been reported.

However, in the present studies, the reconformation of the EA molecules at the interface was not considered to influence the kinetics of the adsorption process, as it has already been shown by the circular dichorism (CD) spectra studies[17] of the adsorption of ovalbumin on ultrafine silica particles that there was hardly any change in the CD spectrum of the ovalbumin after adsorption.

To verify if the adsorption process was diffusion-controlled in our case, we performed experiments at varying speeds of shaking (but mild always) and observed that the adsorbed amount increased with the increasing shaking speed, as shown in Table I. This clearly indicates that the adsorption of EA is also diffusion-controlled. In Figure 4, the process of the adsorption process has been shown as a function of time. It is quite clear from Figure 4 that the rate of adsorption is almost constant by 80 min and, therefore, the kinetic scheme proposed elsewhere[8] may readily be applied. According to the proposed kinetic scheme, if the rate of adsorption remains constant for an appreciable time period, then one can write

$$\frac{d(R_{ad})}{dt} = k_1 c(1 - \theta) - k_2\theta = 0 \qquad (3)$$

From the above eq. (3), expressions for the surface coverage (θ) and the rate constant for the adsorption

Table I Effect of Speed of Shaking on the Adsorption of EA

Speed of Shaking (rpm)	Adsorbed Amount of EA (mg g^{-1}) $\times 10^3$
300	14.2
500	16.8
800	20.0
1000	22.2

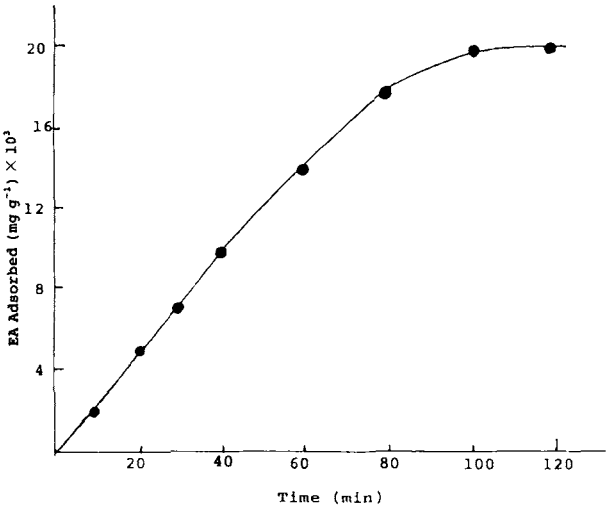

Figure 4 Plot of the amount of EA adsorbed (mg g^{-1}) vs. time at fixed [EA] 6.15 $\times$ 10^{-5} mol dm^{-3}; silica = 0.1 g; pH 11.6; temperature = 25 $\pm$ 0.2°C.

(k_1) may further be derived as described elsewhere[8] in detail.

Evaluation of Rate Constants

Since the adsorption isotherm is of the Langmuir type, obviously, the process of adsorption may be considered as reversible and the rate constants for both the adsorption (k_1) and desorption (k_2) may be calculated. For this purpose, a graph is drawn between $1/c$ and t in accordance with the following equation[8]:

$$\frac{1}{C} = \frac{k_1}{C_0} t + \frac{1}{C_0} \qquad (4)$$

which yields a straight line (Fig. 5) from which the values of the rate constants for the adsorption (k_1) and desorption (k_2) were calculated to be 1.01 $\times$ 10^{-4} s^{-1} and 0.012 $\times$ 10^{-2} mol dm^{-3} s^{-1}, respectively. The superiority of this method for evaluating the rate constants lies in the fact that no complicated mathematical computations (such as in the Runge–Kutta method or Marquardt's optimization routine[18]) are required and only a simple linear plot will serve the purpose well.

However, we also used the Lagergreen equation[19] for the calculation of k_1 according to which

$$\log(q_e - q) = \log q_e - \frac{k_1}{2.303} t \qquad (5)$$

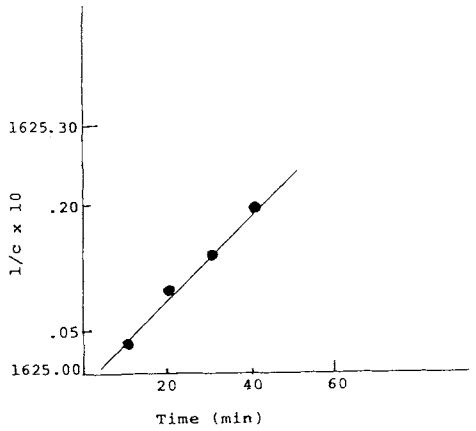

Figure 5 Plot of $1/c$ vs. time for the evaluation of k_1.

where q_e = amount of EA adsorbed at saturation (equilibrium), and q = amount of EA adsorbed at any time t. The plot is shown in Figure 6 and the value of k_1 as calculated from the above equation was found to be 1.41×10^{-4} s^{-1}. It can clearly be seen that the numerical values of the rate constant for the adsorption (k_1) calculated from both methods are almost identical.

Factors Affecting Adsorption

Macromolecular Dimension Effect

A solution of EA was prepared in 0.1*N* NaOH which resulted in a solution of pH 11.6. When H^+ ions were added to this protein solution, almost no change in the pH was noticed, which clearly indicates that the solution was functioning as a true buffer.[20] Due to this reason, we could not study the effect of pH on the adsorption kinetics of EA. It can be seen that by addition of H^+ ions to the protein solution the following equilibrium exists:

$$NH_2CHROO^- + H^+ \rightarrow \overset{+}{N}H_3CHRCOO^- \quad (6)$$

which clearly explains the buffer action of the protein solution. But at the same time due to the formation of dipolar species of the amino acids in the EA solution, a contraction in the dimension of the EA molecules will be produced which should result in a fall in the viscosity of the protein solution. It is very clear from Table II that the reduced viscosity of the protein solution decreases with increasing H^+ ion addition which supports the idea of contraction of the protein molecular dimension. As the dimension of the EA molecule decreases, the adsorption of EA increases, as shown in Table II.

The increase in the adsorption of EA may also be explained in terms of the favorable electrostatic attraction resulting from the added H^+ ions. Since at the experimental pH (11.6) both the EA molecules and the silica surface are negatively charged, the addition of H^+ ions generates dipolar species ($\overset{+}{N}H_3CHCOO^-$), which with their positive ends become attached to the negatively charged silica surface. It is worth mentioning here that in absence of H^+ ions both the EA molecule and silica surface will be negatively charged and then it will be the H-bonding forces operating between the EA molecules and the $-SiO^-$ groups of the silica surface that causes the adsorption of EA molecules.

We also studied the effect of the molecular dimension on the rate of adsorption of EA by monitoring the progress of the adsorption process with respect to time. It was found (figure not shown) that with the decreased viscosity of the EA solution the rate of adsorption also increases. The increase observed may be attributed to the fact that in the medium of lower viscosity the EA molecules will diffuse relatively faster toward the interface and therefore the rate of adsorption increases The rate constants for the adsorption (k_1) at varying reduced viscosity of the medium were calculated and are summarized in Table II.

Salt Effect

The role of ions in affecting adsorption behavior is of great significance as the presence of low molecular weight salts influences the process of adsorption in many ways. For instance, in a polyelectrolyte solution, the quality of solvent decreases in the presence of salts and, consequently, the adsorption increases.[21] Also, in many cases,[22] the small ions screen the electrostatic interactions taking place between the molecules of the adsorbate itself or that between

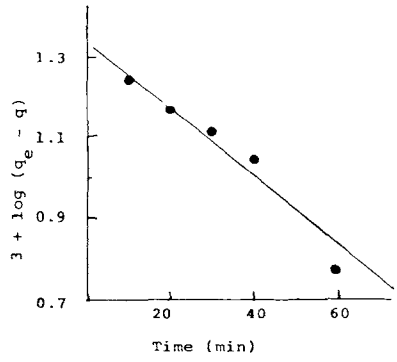

Figure 6 Plot of log ($q_e - q$) vs. time for the evaluation of k_1 according to the Lagergreen equation.

an adsorbate and adsorbent molecule and thus affect the adsorbed amount.

In the present study, the effect of the addition of salts on the amount and rate of the adsorption was studied by adding different salts of the K^+ ion in the concentration range 0.02–0.10*M*. The results are shown in Figure 7, which clearly indicate that the amount of adsorbed EA increases with increasing salt concentration and obeys the following order:

$$Cl^- < SO_4^{-2} < PO_4^{-3}$$

The observed increase seems surprising as at the experimental pH both the EA and silica surface are negatively charged and one has to rule out the possibility of any type of shielding by the added anions. However, the results can be explained as below:

It has been well recognized that several kinds of proteins interact with small molecules and by far the most important contribution to binding is made by albumins.[23] In the present case, at experimental pH 11.6, the protein molecule has a net negative charge, but, nevertheless, it can interact with anions as well as with cations. Every positively or negatively charged group could be considered as a binding site for the small ions[24]; thus, K^+ interacts at the anionic groups (such as $-COO^-$, $-O^-$, and $-S^-$), and anions, at the cationic groups (such as $-NH^+-$, $-NH_3^+$, and $=NH_2^-$). After binding of the added ions to various active sites on the EA molecule, electrostatic repulsions between the EA molecule and the silica surface will be screened, which obviously results in a favorable contact between the EA and the silica surface. Thus, the amount of the adsorbed EA will increase.

It was also observed that the rate of adsorption also increases with the added anions in the same order of effectiveness. The reason for the increased rate is quite clear: Due to a decrease in the electrostatic repulsion between various species in the protein solution, the diffusion of EA molecules will become relatively faster and their attachment to the surface also becomes easier. In this way, the rate of adsorption also increases. The rate constants for adsorption (k_1) have also been calculated and are summarized in Table III.

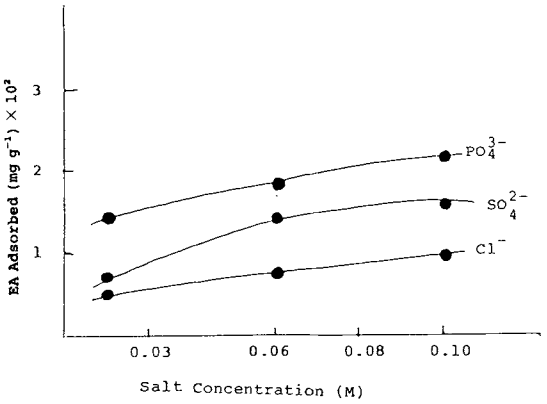

Figure 7 Effect of addition of salts on the plateau adsorption of EA at fixed [EA] = 6.15×10^{-5} mol dm^{-3}; silica = 0.1 g; pH 10.6; temperature = 25 ± 0.2°C.

In an attempt to study the effect of cations on the adsorption, we added Ba^{2+} ions to the solution in the concentration range (0.010–0.020 m) and found that the adsorption decreases with increasing Ba^{2+} concentration. This is due to the preferential adsorption of Ba^{2+} ions on the negative-charged surface.

Effect of Additives

The influence of the presence of foreign species in the protein solution on the adsorption behavior of the EA has been investigated by carrying out adsorption experiments in the presence of the cationic and anionic surfactants.

Surfactant Effect

The effect of the cationic surfactant on the adsorption of the EA was studied by adding cetyl trimethylammonium bromide (CTAB) in the concentration

Table II Effect of Viscosity of the EA Solution on Its Adsorption

Volume of Concn HCl Added (in mL) to 20 mL EA Solution (mL)	Reduced Viscosity η_{red} (dm g^{-1})	Adsorbed Amount of EA (mg g^{-1})	Rate Constant of Adsorption (k_1) × 10^4 s^{-1}
0.0	1.07	0.02	1.01
0.2	0.83	0.05	2.18
0.4	0.68	0.06	2.90
0.6	0.41	0.07	3.30
0.8	0.27	0.08	3.88

range 2.0–6.0 × 10^{-4} mol dm^{-3}. The results are shown in Figure 8, which imply that both the adsorbed amount and adsorption rate increase with the increasing concentration of the surfactant. The reason for the observed increase is quite apparent as the added cationic surfactant may bind to the negatively charged sites on the EA molecule and thus reduce the electrostatic repulsion between the EA molecules and silica surface which finally increases the adsorbed mass.

One more aspect of the detergent addition is that it may cause coagulation of the EA which as a consequence results in unfolding of the protein chains. Obviously, due to the unfolding of the EA molecules, a greater number of active sites will be exposed to the adsorbent surface and, therefore, the adsorbed mass will increase.

For studying the effect of the anionic surfactant, sodium oleate was added in the concentration range of 2.0–6.0 × 10^{-4} mol dm^{-3} and the results are shown in Figure 8. It is clear from Figure 8 that both the adsorbed mass and the adsorption rate decrease on increasing the concentration of sodium oleate in the studied range. The results may be attributed to the fact that the presence of anionic surfactant molecules in the protein solution cause the electrostatic repulsion to increase which results in a decreased adsorption.

Temperature Effect

The influence of temperature on the adsorption of EA was studied by carrying out adsorption experiments in the temperature range 5–45°C. Results are shown in Figure 9, which clearly indicate that the adsorbed amount decreases on increasing the temperature and, finally, it becomes zero at 45°C. The

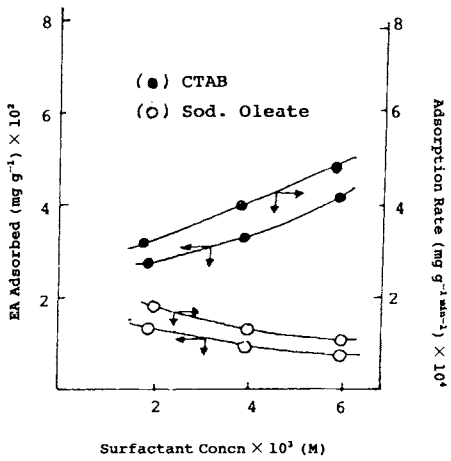

Figure 8 Effect of addition of surfactants on the plateau adsorption of EA at fixed [EA] = 6.15 × 10^{-5} mol dm^{-3}; silica = 0.1 g; pH 11.6; temperature = 25 ± 0.2°C.

observed temperature dependence of adsorption is well expected also as at higher temperatures. Van der waals forces, which are solely responsible for the observed adsorption, decrease, and as a consequence, the adsorption decreases. Similar results have also been obtained elsewhere.[25,26] However, in several investigations,[27] an increase in temperature has resulted in a greater adsorption, which may be explained by the fact that in several adsorbents the number of active sites increase with increasing temperature and, therefore, the adsorption increases.

CONCLUSIONS

The adsorption of egg albumin (EA) onto the silica surface follows the Langmuir adsorption isotherm

Table III Effect of Addition of Salts on the Rate Constants of Adsorption (k_1)

Salt	Concentration (M)	Rate Constant (k_1) × 10^4 s^{-1}	Adsorption Rate (mg g^{-1} min^{-1}) × 10^4
KCl	0.02	1.88	4.8
	0.06	3.12	7.0
	0.10	3.84	9.6
K_2SO_4	0.02	3.20	7.2
	0.06	4.76	15.4
	0.10	4.92	15.4
K_3PO_4	0.02	6.36	15.0
	0.06	8.82	10.8
	0.10	9.48	24.6
No salt	—	1.01	2.5

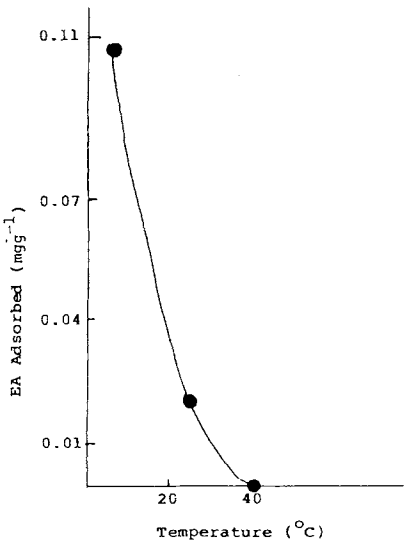

Figure 9 Effect of temperature (°C) on the plateau adsorption of EA at fixed [EA] = 6.15×10^{-5} mol dm^{-3}; silica = 0.1 g; pH 11.6.

equation and belongs to the L2 type of the adsorption isotherm. The adsorption rate is found to remain almost constant up to 80 min and then it levels off. Both the amount of adsorbed EA and the rate constant of adsorption increase with the decreasing reduced viscosity of the medium. In the same way, both the adsorption rate and rate constant of adsorption increase with the increasing concentration and the charge of the added anions. In the case of the addition of surfactant to the adsorption medium, it is found that both the adsorbed amount and the adsorption rate increase with the increasing cationic surfactant (CTAB) concentration. On the other hand, with the increasing anionic surfactant (sodium oleate), the amount adsorbed and the adsorption rate are found to decrease. The adsorption is quite sensitive to the temperature and decreases with increasing temperature.

REFERENCES

1. S. L. Cooper, in *Interaction of the Blood with Natural and Artificial Surfaces,* E. W. Sattzman, Ed., Marcel Dekker, New York, 1981.
2. J. D. Andrade, in *Surface and Interfacial Aspects of Biomedical Polymers,* J. D. Andrade, Ed., Plenum, New York, 1985, Vol. 2.
3. J. L. Brash and T. A. Horbett, Eds., *Proteins at Interfaces, Physiochemical and Biomedical Studies,* ACS Symposium Series 343, American Chemical Society, Washington, DC, 1987.
4. J. C. Dijit, M. A. Cohen Stuart, J. E. Hofman, and G. J. Fleer, *Colloids Surf.,* **51,** 141 (1990).
5. A. V. Elgersma, R. L. J. Zsom, J. Lyklema, and W. Norde, *Colloids Surf.,* **65,** 17 (1992).
6. J. D. Aptel, J. C. Voegel, and A. Schmitt, *Colloids Surf.,* **29,** 359 (1988).
7. U. D. N. Bajpai and A. K. Bajpai, *Polym. Int.,* **32,** 43 (1993).
8. A. K. Bajpai, *J. Appl. Polym. Sci.,* **51,** 651 (1994).
9. A. K. Bajpai and S. K. Bajpai, *Colloids Surf.,* **101,** 21 (1995).
10. A. K. Bajpai and S. K. Bajpai, *Colloid Polym. Sci.,* **273,** 1028 (1995).
11. A. K. Bajpai, *Polym. Int.,* **33,** 315 (1994).
12. A. K. Bajpai, *J. Macromol. Sci. Pure Appl. Chem. A,* **32**(3), 467 (1995).
13. A. K. Bajpai and M. Rajpoot, *Bull. Chem. Soc.* (Jpn.), to appear.
14. A. Kaplan and L. L. Szabo, in *Clinical Chemistry: Interpretation and Techniques,* Lea and Febiger, Philadelphia, 1987, p. 157.
15. C. H. Giles, T. H. Macewan, S. N. Nakhuwa, and D. Smith, *J. Chem. Soc. Faraday Trans.,* **4,** 3973 (1960).
16. S. Chibowski, *J. Colloid Interf. Sci.,* **140,** 444 (1990).
17. A. Kondo, S. Oku, and K. Higashitani, *J. Colloid Interf. Sci.,* **143,** 214 (1991).
18. D. D. Ravethar, V. D. Ambeskar, and R. A. Mashelker, *J. Appl. Polym. Sci.,* **39,** 769 (1990).
19. B. K. Singh and N. S. Rawat, *J. Chem. Tech. Biotechnol.,* **61,** 57 (1994).
20. A. L. Lehniner, *Principles of Biochemistry,* CBS, New Delhi, 1984.
21. A. Eisenberg and O. King, in *Ion Containing Polymers,* C. R. S. Stein, Ed., Academic Press, New York, 1977, Vol. 2, Chap. V.
22. F. Cosawa, *Polyelectrolytes,* Marcel Dekker, New York, 1971.
23. A. Goldstein, L. Aronow, and S. M. Kalman, *Principles of Drug Action: The Basis of Pharmacology,* Wiley, New York, 1974.
24. C. Tanford, S. A. Swanson, and W. S. Shore, *J. Am. Chem. Soc.,* **77,** 6414 (1955).
25. G. S. Gupta, S. P. Shukla, G. Prasad, and V. N. Singh, *Environ. Technol.,* **13,** 925 (1992).
26. V. R. Choudhary, S. N. Sausare, and G. A. Thite, *J. Chem. Tech. Biotechnol.,* **42,** 249 (1988).
27. G. Mckay, M. S. Otterburn, and A. G. Sweeney, *Water Res.,* **14,** 15 (1980).

Received June 23, 1995
Accepted December 5, 1995